FEBS Letters 588 (2014) 4364–4368

journal homepage: www.FEBSLetters.org

O_2 reduction by photosystem I involves phylloquinone under steady-state illumination

Marina A. Kozuleva [a,*], Anastasia A. Petrova [b], Mahir D. Mamedov [a,b], Alexey Yu. Semenov [b], Boris N. Ivanov [a]

[a] Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Russia
[b] A.N. Belozersky Institute of Physical-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia

ARTICLE INFO

Article history:
Received 8 July 2014
Revised 13 September 2014
Accepted 1 October 2014
Available online 13 October 2014

Edited by Richard Cogdell

Keywords:
Photosynthesis
Electron transfer
Photosystem I
Oxygen reduction
Phylloquinone
Plastoquinone

ABSTRACT

O_2 reduction was investigated in photosystem I (PS I) complexes isolated from cyanobacteria *Synechocystis* sp. PCC 6803 wild type (WT) and *menB* mutant strain, which is unable to synthesize phylloquinone and contains plastoquinone at the quinone-binding site A_1. PS I complexes from WT and *menB* mutant exhibited different dependencies of O_2 reduction on light intensity, namely, the values of O_2 reduction rate in WT did not reach saturation at high intensities, in contrast to the values in *menB* mutant. The obtained results suggest the immediate phylloquinone involvement in the light-induced O_2 reduction by PS I.

1. Introduction

Molecular oxygen, O_2, is involved in numerous biochemical reactions in aerobic cells. In photosynthetic organisms, O_2 can interact with the photosynthetic apparatus, decreasing the quantum yield of CO_2 fixation. Moreover, as a result of such reactions, reactive oxygen species (ROS) are produced. During evolution, the photosynthetic apparatus has been optimized to minimize reactions with O_2 [1,2], although they still occur and may have a positive impact on cellular metabolism [3]. One such reaction, the so-called Mehler reaction, represents O_2 reduction by components of the photosynthetic electron transfer chain (ETC) under illumination [4]. Electron flow to O_2 was shown to occur along with electron transfer (ET) to $NADP^+$ [5,6]. Under conditions of limited NADPH utilization and excessive light, the Mehler reaction prevents over-reduction of the ETC and photoinhibition [7,8]. Moreover, a stable product of the Mehler reaction, H_2O_2, might be a signal messenger indicating ETC functional state [9,10].

The exact components of the ETC involved in O_2 reduction in vivo have not been yet clearly identified. Ferredoxin (Fd), an acceptor of electrons from Photosystem I (PS I), used to be considered as the main O_2 reducing agent [11], although this assumption was challenged [12,13]. Recently, it was shown that reduced Fd was only capable of low rates of O_2 reduction in the presence of $NADP^+$ and its contribution to the total O_2 reduction was 5–10% [14]. This implies that thylakoid membrane-bound components play an essential role in O_2 reduction. Redox-cofactors of PS I are believed to be significant O_2 reducing agents [3,15]. The ETC of PS I consists of the primary electron donor (P_{700}), the primary (A_0), and secondary (A_1, F_X, F_A, and F_B) electron acceptors (Fig. 1A). Under single-flash illumination, the terminal electron cofactors of PS I, the [4Fe–4S] clusters $(F_A/F_B)^-$, are oxidized by O_2 in the absence of Fd [16]. This reaction results in production of the superoxide anion-radical ($O_2^{\cdot-}$) outside the thylakoid membrane. Using the EPR-detector 1-hydroxy-4-isobutyramido-

Abbreviations: DMF, dimethylformamide; ET, electron transfer; ETC, electron transfer chain; Fd, ferredoxin; MV, methyl viologen; PS I, photosystem I; PhQ and $PhQ^{\cdot-}$, phylloquinone and phyllosemiquinone; PQ and $PQ^{\cdot-}$, plastoquinone and plastosemiquinone; ROS, reactive oxygen species; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; TMT-H, 1-hydroxy-4-isobutyramido-2,2,6,6-tetramethylpiperidinium; V_{ET}, $V_{ET}^{O_2}$ and V_{ET}^{MV}, rates of electron transfer as such and to O_2 and MV, respectively; WT, wild type

* Corresponding author at: Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow region 142290, Russia. Fax: +7 4967 330532.

E-mail address: kozuleva@gmail.com (M.A. Kozuleva).

http://dx.doi.org/10.1016/j.febslet.2014.10.003

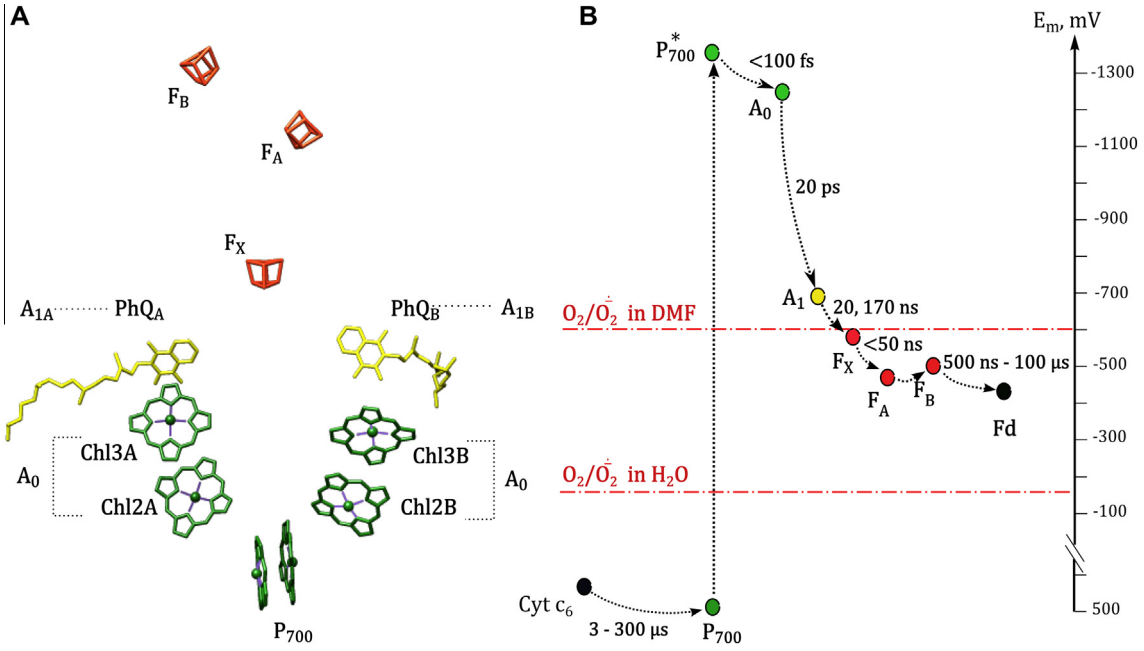

Fig. 1. (A) Arrangement of electron transfer cofactors in PS I. (B) Diagram of forward electron transfer in cyanobacterial PS I with lifetimes and E_m values of the cofactors. E_m values of ($O_2/O_2^{\cdot-}$) in water and dimethylformamide (DMF) are also shown.

2,2,6,6-tetramethylpiperidinium (TMT-H), $O_2^{\cdot-}$ was directly shown to be produced within as well as outside the membrane under steady-state illumination of isolated thylakoids [17,18]. This allowed assuming that at least two cofactors reduce O_2 simultaneously. This assumption is supported by the study of apparent K_m of spinach PS I for O_2 that revealed the existence of at least two sites of O_2 reduction [19]. In the lipid bilayer of the membrane, the midpoint redox-potential (E_m) of ($O_2/O_2^{\cdot-}$) is close to that in dimethylformamide (−500 ÷ −600 mV *vs.* NHE) [20]. Phylloquinones (PhQ), which occupy the quinone-binding sites of PS I (A_1-sites), and the [4Fe–4S] cluster F_X are characterized by rather negative E_m values and are capable of O_2 reduction within the membrane (Fig. 1B). PhQ [21,22] and F_X [23] were proposed to be O_2 reducing cofactors, however there is no direct evidence proving their involvement in O_2 reduction.

In order to investigate the possible role of phyllosemiquinone ($PhQ^{\cdot-}$) at the A_1-site in O_2 reduction, we used PS I isolated from the wild type (WT) and PhQ-lacking mutant strain of *Synechocystis* sp. PCC 6803 with the gene encoding 1,4-hydroxynaphthoyl-CoA-synthase (*MenB*) knocked out [24,25]. In mutant, plastoquinone (PQ) occupies the A_1-sites, with E_m ($PQ/PQ^{\cdot-}$) ~100 mV more positive than E_m ($PhQ/PhQ^{\cdot-}$) in PS I from WT [26]. In this study, it was found that PS I complexes from the WT and *menB* mutant exhibited different dependencies in O_2 reduction on light intensity, and this was interpreted as evidence for direct PhQ involvement in O_2 reduction.

2. Methods

Cells of *Synechocystis* sp. PCC 6803 wild type and mutant strains were grown in BG-11 medium at 30 °C under white fluorescent illumination. PS I complexes were isolated as described in [27]. Reaction medium contained 50 mM HEPES–KOH (pH 7.6), 20 mM NaCl, 5 mM $MgCl_2$, 0.03% β-dodecyl-*n*-maltoside; *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) and sodium ascorbate were added as artificial electron donors to photooxidized P_{700}^+. Redox state of P_{700} was determined by difference in absorption at 810–860 nm with PAM101 equipped with EDP700DW module. A suspension of PS I was placed in a standard chamber (WALZ, Germany) and illuminated by the white light of an incandescent lamp (KL-1500) for 30 s. Light intensity was varied by altering voltage. Light-induced steady-state rate of O_2 reduction was measured as O_2 uptake with Clark type O_2-electrode under stirring at 22 °C. Suspension of PS I was placed in a custom-build thermostatic chamber and illuminated by LED with maximum emission at 660 nm for 1 min. Intensity of the LED was varied by neutral filters or altering electric current. The steady-state rates of ET from PS I to O_2 were proportional to the rates of O_2 uptake because a single PS I turnover resulted in generation of $O_2^{\cdot-}$. In the presence of methyl viologen (MV), a single PS I turnover also resulted in $O_2^{\cdot-}$ generation via the radical form of MV with the rate constant equal to 8×10^8 M^{-1} s^{-1} [28]. Superoxide radicals further produce H_2O_2 in the reaction with ascorbate, present in the suspension at high (5–10 mM) concentration, thereby suppressing spontaneous dismutation of $O_2^{\cdot-}$. The resulting stoichiometry was 1 electron per 1 O_2 molecule consumed [29], and, in our experiments, it was confirmed by addition of superoxide dismutase and catalase. Thus, light-induced O_2 uptake was used as readout of O_2 reduction in the absence and presence of MV. Light intensity as photon flux density µE m^{-2} s^{-1} was determined using a LI-COR model LI-250 quantum meter (Licor, Nebraska, USA).

TMPD-2HCl, sodium ascorbate, MV, β-dodecyl-*n*-maltoside were purchased from Sigma and AppliChem.

3. Results

Steady-state illumination of isolated PS I complexes resulted in O_2 reduction (Fig. 2). In order to accurately study the mechanism of O_2 reduction by PS I under steady-state illumination, this reaction must be the overall ET limiting step. In contrast to O_2, MV is a very efficient acceptor of electrons from the terminal cofactors of PS I, with the rate constant of reduction equal to 10^7 M^{-1} s^{-1} [30]. In the presence of MV, the rate of ET (V_{ET}) is equal to the rate of MV reduction (V_{ET}^{MV}), and this rate is limited by electron donation to P_{700}^+ [31]. The comparison of the rate of ET to O_2 ($V_{ET}^{O_2}$) and V_{ET}^{MV} is a useful approach to reveal the rate-limiting step. The addition

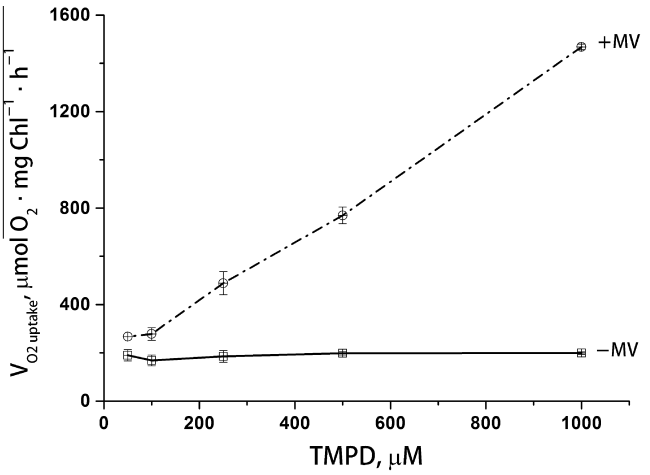

Fig. 2. Effect of TMPD concentration on rates of electron transfer (V_{ET}) in the absence and in the presence of methyl viologen (MV). PS I^{WT}, 2 μg Chl ml^{-1}, 10 mM ascorbate, 100 μM MV, if present. Light intensity, 650 μE m^{-2} s^{-1}.

of MV led to both stimulation of V_{ET} (Fig. 2) and increase in the light-induced steady-state level of P^{+}_{700} (ΔP^{+}_{700}; Fig. 3). Values of V^{MV}_{ET} were directly proportional to TMPD concentration (Fig. 2), in contrast to $V^{O_2}_{ET}$ which was not affected by an increase in TMPD concentration from 50 μM to 1.0 mM. These results indicated that, in the absence of MV, O_2 reduction was indeed the rate-limiting step, and that TMPD can be used for the study of light-induced steady-state ET from isolated PS I to O_2 measured as O_2 consumption.

In PS I isolated from WT (PS I^{WT}), an increase in light intensity led to a rise of both ΔP^{+}_{700} (Fig. 3) and $V^{O_2}_{ET}$ (Fig. 4A). The values of these parameters did not reach saturation over the range of light intensities tested. The stimulation of V_{ET} by MV was observed at all light intensities (Fig. 4A). With increasing light intensity, the stimulation diminished, therefore the ratio of V^{MV}_{ET} to $V^{O_2}_{ET}$ (R^{MV}), significantly decreased (Fig. 4C).

The $V^{O_2}_{ET}$ values in PS I^{WT} and PS I isolated from the *menB* mutant (PS I^{menB}) were similar at low and moderate light intensities (Fig. 4A *vs.* B). With increasing light intensity, values of $V^{O_2}_{ET}$ in PS I^{menB} increased up to the steady-state level at light intensities above 650 μE m^{-2} s^{-1} (Fig. 4B). It was previously shown that strong light treatment of PS I^{menB} caused the irreversible blockage of ET from A_1 to F_X due to double PQ reduction [32]. In our experiments, pre-illumination of the suspension of either PS I^{WT} or

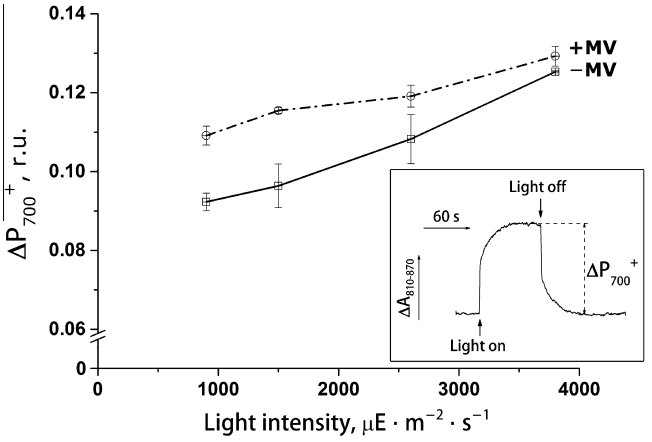

Fig. 3. Effect of light intensity on light-induced steady-state level of P^{+}_{700} (ΔP^{+}_{700}). PS I^{WT}, 5 μg Chl ml^{-1}, 10 mM ascorbate, 1 mM TMPD, 100 μM MV, if present. Insert: typical kinetics of light-induced P_{700} redox-changes.

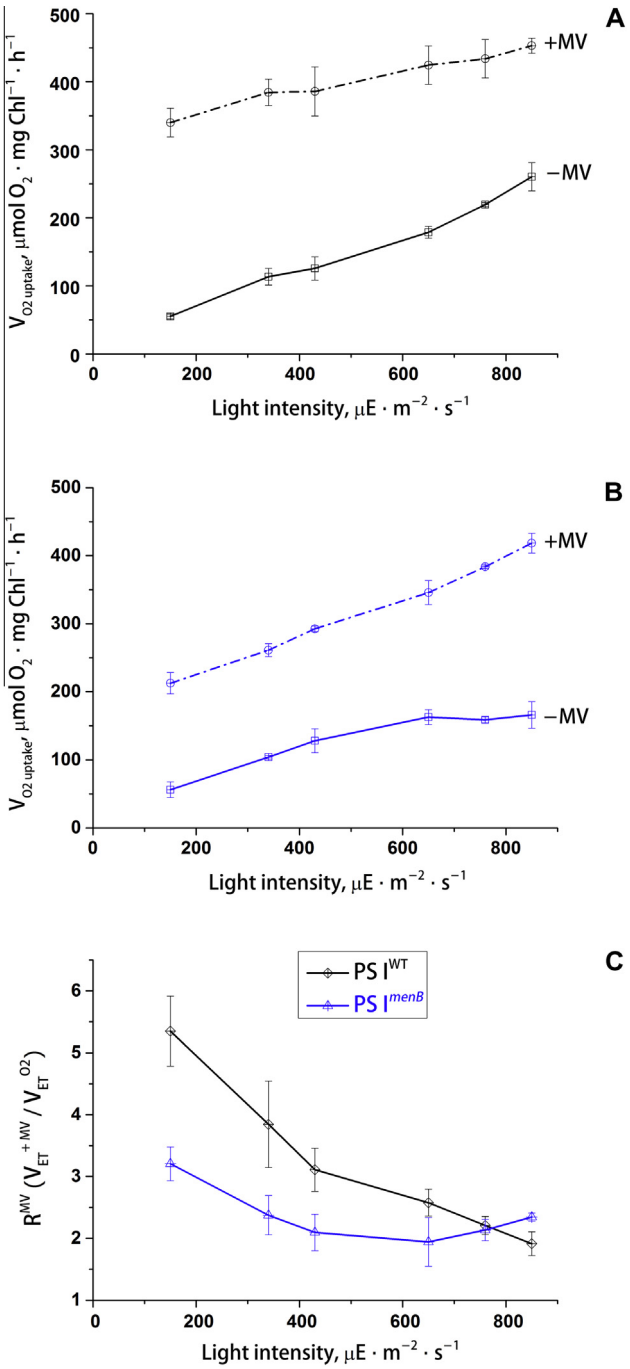

Fig. 4. Effect of light intensity on steady-state rates of O_2 uptake in the absence and in the presence of methyl viologen (MV) in suspension of either PS I^{WT} (A) or PS I^{menB} (B). The results were reproduced with 3 independent PS I^{WT} and PS I^{menB} preparations. (C) Effect of light intensity on R^{MV}; the mean values of 5 individual experiments are presented. PS I, 2 μg Chl ml^{-1}, 5 mM ascorbate, 250 μM TMPD, 100 μM MV, if present.

PS I^{menB} by high light for 1 min in the absence of MV did not affect the V^{MV}_{ET} measured after addition of MV to the suspension (data not shown). This indicated that the total ET to F_A/F_B was not irreversibly blocked in either PS I^{menB} or PS I^{WT} in the absence of MV.

Similarly to PS I^{WT}, an addition of MV to the suspension of PS I^{menB} led to an increase in V_{ET}. The effect of MV showed that, in the absence of MV, the total rate of ET in PS I^{menB} was also limited at the level of O_2 reduction by cofactors of PS I. V^{MV}_{ET} values in both PS I^{WT} and PS I^{menB} were directly proportional to light

intensity. In high light, the values of V_{ET}^{MV} were similar in both types of complexes. This might indicate that steady-state ET to an efficient acceptor like MV was slightly affected by replacement of PhQ by PQ under saturating conditions.

R^{MV} in PS I^{menB} decreased to a certain low level at high light intensities in contrast to PS I^{WT} (Fig. 4C). This clearly reveals a difference in the peculiarities of O_2 reduction by PS I from WT *vs.* *menB* mutant.

4. Discussion

The results presented here are in line with previous data [17–19,21] and provide evidence of an operation of at least two pathways of O_2 reduction by isolated PS I. Obviously, under steady-state illumination of isolated PS I in the absence of Fd or MV, the terminal cofactors $(F_A/F_B)^-$ are oxidized by O_2. One may expect that additional O_2 reduction by PS I can be significant under conditions of limited oxidation of $(F_A/F_B)^-$ by an inefficient electron acceptor such as O_2. O_2 reduction at the A_1-site is the most plausible explanation for O_2^- production within the thylakoid membrane [17]. Increasing light intensity was shown to accelerate O_2^- production within the membrane rather than in water bulk phase [18]. This is in line with the higher involvement of $PhQ^{\cdot-}$ in O_2 reduction under high light conditions (Fig. 4).

The dependence of $V_{ET}^{O_2}$ in PS I^{menB} on light intensity exhibited a saturation behavior at high light intensities (Fig. 4B), in contrast to proportional increase in $V_{ET}^{O_2}$ values in PS I^{WT}. What could be a cause of this saturation? Besides a possible irreversible blockage of ET from A_1 to F_X in PS I^{menB}, which was excluded by the comparison of V_{ET}^{MV} measured before and after illumination of the complexes in the absence of MV (see Section 3), the saturation in PS I^{menB} could be explained as a result of either decrease of the rate of O_2 reduction by $(F_A/F_B)^-$ due to retardation of ET from $PQ^{\cdot-}$ to the [4Fe–4S] clusters in high light, or saturation of all pathways of O_2 reduction in PS I^{menB}.

The addition of MV demonstrated that the overall rates in both types of complexes were limited by ET to O_2. It means that neither electron donation to P_{700}^+ nor ET between cofactors of PS I are rate-limiting steps of the total ET in PS I^{menB} as well as in PS I^{WT}. Since PhQ substitution by PQ does not affect characteristics of ET from F_X to F_A/F_B, the rates of $(F_A/F_B)^-$ oxidation by O_2 could be different in PS I^{WT} and PS I^{menB} only because of different rates of ET to F_A/F_B. At a first approximation, V_{ET}^{MV} reflects the rate of F_A/F_B reduction in PS I under given conditions. The stimulation of V_{ET} by MV and the similar values of V_{ET}^{MV} in PS I^{WT} and PS I^{menB} at high light intensities imply that, in the absence of MV, ET from P_{700} to the [4Fe–4S] clusters in PS I^{menB} is not retarded in comparison with such ET in PS I^{WT}. In order to take into account a contribution of O_2 reduction by $(F_A/F_B)^-$ to the total O_2 reduction, a relationship between V_{ET}^{MV} and $V_{ET}^{O_2}$ (the R^{MV} value) was calculated. The shapes of the dependencies of R^{MV} on light intensity were different in PS I^{WT} and PS I^{menB} (Fig. 4C). It indicates that there is a difference in characteristics of O_2 reduction by PS I from WT *vs.* *menB* mutant. The results imply that both $PhQ^{\cdot-}$ and $PQ^{\cdot-}$ at the A_1-site serve as O_2 reducing agents but with different ability.

The steady-state rate of O_2 reduction by semiquinone at the A_1-site depends both on the rate constant of the reaction and the quasi-steady-state concentration of semiquinone in PS I. Under continuous illumination, the steady-state level of $PhQ^{\cdot-}$ was measured in isolated spinach thylakoids and cyanobacteria cells [33]. The single flash-induced ET from semiquinone to F_X in the PS I from *menB* mutant is ~1000 times slower than in the WT because the E_m value $(PQ/PQ^{\cdot-})$ at the A_1-site is ~100 mV more positive than that of $(PhQ/PhQ^{\cdot-})$ [26]. The difference in E_m can also lead to a significant difference in rates of O_2 reduction by $PhQ^{\cdot-}$ and $PQ^{\cdot-}$. Since O_2 reduction by $PhQ^{\cdot-}$ is more thermodynamically favorable than O_2 reduction by $PQ^{\cdot-}$, then the rate constant for $PhQ^{\cdot-}$ is much higher than for $PQ^{\cdot-}$. Owing to the lower rate of $PQ^{\cdot-}$ oxidation by both F_X and O_2, the quasi-steady-state concentration of $PQ^{\cdot-}$, providing saturation of its oxidation by O_2, should be reached at lower light intensities than that of $PhQ^{\cdot-}$. This is in line with the saturation behavior of $V_{ET}^{O_2}$ in PS I^{menB} in contrast to the rise of $V_{ET}^{O_2}$ in PS I^{WT} (Fig. 4A *vs.* 4B).

Thus, E_m of the quinone/semiquinone pair at the A_1-site can be the determining factor of the semiquinone reaction with O_2. ET cofactors from P_{700} to F_X are located along pseudo-C_2 symmetry axis forming A- and B-branches. Both branches are capable of transferring electrons [34], although with non-equal contributions, at least in PS I from cyanobacteria [35,36]. The speculations about possible advantages of maintaining a minor ET activity of the B-branch ascribe certain safety roles to the B-branch [1,37]. One of the most essential distinctions between the two branches is the kinetics of ET from A_1 to F_X due to the difference in E_m values of $(PhQ/PhQ^{\cdot-})$ in A_{1A}- and A_{1B}-sites, −671 and −844 mV, respectively [38]. These E_m values provide quite high ΔG^0 for the reaction with O_2 within the protein, with O_2 reduction by $PhQ^{\cdot-}$ at the A_{1B}-site being more thermodynamically favorable than that of $PhQ^{\cdot-}$ at the A_{1A}-site. Under steady-state illumination in the presence of efficient electron donor to P_{700}^+ and restricted outflow of electrons from PS I, charge separation could occur sequentially in both branches of PsaA/PsaB heterodimer, resulting in the production of two $PhQ^{\cdot-}$ per one F_X. It is possible that under these conditions, the higher ΔG^0 for O_2 reduction by $PhQ^{\cdot-}$ in the B-branch provides the preferential O_2 reduction via the B-branch, while the ET to F_X and further to F_A/F_B occurs predominantly through the A-branch. In this case, $PhQ^{\cdot-}$ oxidation by O_2 could support the overall ET preventing charge recombination that could lead to triplet P_{700}^* formation and hence possible singlet O_2 ($^1O_2$) production [1].

It is interesting that mutants of cyanobacteria with blocked biosynthesis of PhQ were shown to lose the ability to photoautotrophic growth in high light and reveal a 40% reduction of growth in low light [24,39]. Negative effect of PhQ deficiency on growth and light sensitivity were detected for other organisms, namely *menD1* mutant of the green algae *Chlamydomonas reinhardtii* with PQ at the A_1-site [40] and *AtmenG* mutant of *Arabidopsis thaliana* with demethylphylloquinone at the A_1-site [41]. However, the activity of PS I from the mutants under steady-state illumination measured in the presence of efficient electron donors to P_{700} and efficient electron acceptors (MV or flavodoxin) differed only slightly from the WT [24,40,41]. It seems unlikely that the sensitivity of these mutants to high light is caused by slowing down ET from A_1 to F_X. Perhaps, some mechanisms of protection against photoinhibition are suppressed in these mutants. The Mehler reaction is considered to be an essential mechanism for protecting the photosynthetic apparatus against photoinhibition in high light [15]. Our results indicate that one of the mechanisms impaired in *menB* mutant can be O_2 reduction by semiquinone at the A_1-site.

Fe–S proteins are considered as inefficient O_2 reducing agents [21]. The rate constant of the reaction of O_2 with reduced Fd was $10^3\ M^{-1}\ s^{-1}$ [13,22,42], while O_2 reduction by semiquinones possessing E_m $(Q/Q^{\cdot-})$ close to E_m (Fd/Fd^-) was approximately $10^9\ M^{-1}\ s^{-1}$ [20]. In vivo, high rates of $(F_A/F_B)^-$ oxidation by O_2 are undesirable for photosynthetic organisms because efficient flow of electrons from $(F_A/F_B)^-$ to O_2 would prevent Fd reduction [2]. It was proposed that uphill ET from F_A to F_B in PS I was evolutionary adaptation to minimize O_2 reduction by F_B in the absence of oxidized Fd [43]. In isolated PS I complexes in the absence of electron acceptors such as Fd or MV, the reaction of $(F_A/F_B)^-$ with O_2 is unavoidable under continuous illumination. Thus, essential $(F_A/F_B)^-$ oxidation by O_2 can be a result of the absence of Fd. In this

study, PhQ involvement in O_2 reduction under continuous illumination of PS I complexes has been shown to occur along with ET to [4Fe–4S] clusters. Thus, the reaction of O_2 with $PhQ^{\cdot-}$ can be the major mechanism of O_2 reduction by PS I in vivo. Since PS I is considered as the main site of the Mehler reaction, the results presented in this study support the idea that O_2 reduction by $PhQ^{\cdot-}$ makes an essential contribution to the Mehler reaction in vivo.

Acknowledgements

Authors are thankful to Dr. G.T. Hanke (Osnabrueck University, Germany) and Dr. A.N. Tikhonov (MSU, Russia) for valuable discussion. The *menB* mutant strain of *Synechocystis* sp. PCC 6803 was kindly provided by Dr. J.H. Golbeck (Pennsylvania State University, USA). The reported study was supported by Russian Foundation for Basic Research, Research Projects 12-04-31219, 12-04-00320, 12-04-00821, 14-04-00519 and 13-04-40299-H.

References

[1] Rutherford, A.W., Osyczka, A. and Rappaport, F. (2012) Back-reactions, short-circuits, leaks and other energy wasteful reactions in biological electron transfer: redox tuning to survive life in O_2. FEBS Lett. 586, 603–616.
[2] Jagannathan, B., Shen, G. and Golbeck, J.H. (2012) The evolution of type I reaction centers: the response to oxygenic photosynthesis in: Functional Genomics And Evolution Of Photosynthetic Systems. Advances in Photosynthesis and Respiration 33 (Burnap, R.L. and Vermaas, W.F.J., Eds.), pp. 285–316, Springer, Netherlands.
[3] Ivanov, B., Kozuleva, M. and Mubarakshina, M. (2012) Oxygen metabolism in chloroplast in: Cell Metabolism – Cell Homeostasis and Stress Response (Bubulya, P., Ed.), pp. 39–72, InTech.
[4] Mehler, A.C. (1951) Studies on reactions of illuminated chloroplasts. I. Mechanism of the reduction of oxygen and other Hill reagents. Arch. Biochem. Biophys. 33, 65–77.
[5] Allen, J.F. (1975) Oxygen reduction and optimum production of ATP in photosynthesis. Nature 256, 599–600.
[6] Furbank, R.T. and Badger, M.R. (1983) Oxygen exchange associated with electron transport and phosphorylation in spinach thylakoids. Biochem. Biophys. Acta 723, 400–409.
[7] Park, Y.-I., Chow, W.S., Osmond, C.B. and Anderson, J.M. (1996) Electron transport to oxygen mitigates against the photoinactivation of Photosystem II in vivo. Photosynth. Res. 50, 23–32.
[8] Ort, D.R. and Baker, N.R. (2002) A photoprotective role for O_2 as an alternative electron sink in photosynthesis? Curr. Opin. Plant Biol. 5, 193–198.
[9] Mubarakshina, M.M., Ivanov, B.N., Naydov, I.A., Hillier, W., Badger, M.R. and Krieger-Lizkay, A. (2010) Production and diffusion of chloroplastic H_2O_2 and its implication to signaling. J. Exp. Bot. 61, 3577–3587.
[10] Miller, G., Shulaev, V. and Mittler, R. (2008) Reactive oxygen signaling and abiotic stress. Physiol. Plant. 133, 481–489.
[11] Robinson, J.M. (1988) Does O_2 photoreduction occur within chloroplasts in vivo? Physiol. Plant. 72, 666–680.
[12] Asada, K., Kiso, K. and Yoshikawa, K. (1974) Univalent reduction of molecular oxygen by spinach chloroplasts on illumination. J. Biol. Chem. 249, 2175–2181.
[13] Golbeck, J. and Radmer, R. (1984) Is the rate of oxygen uptake by reduced ferredoxin sufficient to account for photosystem I – mediated O2 reduction? in: Advances in Photosynthesis Research (Sybesma, C, Ed.), M. Nijhoff/Dr W. Junk Publ, The Hague/Boston/Lancaster. 1.4.561–1.4.564.
[14] Kozuleva, M.A. and Ivanov, B.N. (2010) Evaluation of the participation of ferredoxin in oxygen reduction in the photosynthetic electron transport chain of isolated pea thylakoids. Photosynth. Res. 105, 51–61.
[15] Asada, K. (1999) The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Ann. Rev. Plant Physiol. Plant Mol. Biol. 50, 601–639.
[16] Vassiliev, I.R., Jung, Y.S., Yang, F. and Golbeck, J.H. (1998) PsaC subunit of photosystem I is oriented with iron-sulfur cluster F_B as the immediate electron donor to ferredoxin and flavodoxin. Biophys. J. 74, 2029–2035.
[17] Kozuleva, M., Klenina, I., Proskuryakov, I., Kirilyuk, I. and Ivanov, B. (2011) Production of superoxide in chloroplast thylakoid membranes. ESR study with cyclic hydroxylamines of different lipophilicity. FEBS Lett. 585, 1067–1071.
[18] Mubarakshina-Borisova, M.M., Kozuleva, M.A., Rudenko, N.N., Naydov, I.A., Klenina, I.B. and Ivanov, B.N. (2012) Photosynthetic electron flow to oxygen and diffusion of hydrogen peroxide through the chloroplast envelope via aquaporins. Biochim. Biophys. Acta 1817, 1314–1321.
[19] Lien, S. and San Pietro, A. (1979) On the reactivity of oxygen with photosystem I electron acceptors. FEBS Lett. 99, 189–193.
[20] Wardman, P. (1990) Bioreductive activation of quinones: redox properties and thiol reactivity. Free Radical Res. Commun. 8, 219–229.
[21] Kruk, J., Jemioła-Rzemińska, M., Burda, K., Schmid, G.H. and Strzałka, K. (2003) Scavenging of superoxide generated in photosystem I by plastoquinol and other prenyllipids in thylakoid membranes. Biochemistry 42, 8501–8505.
[22] Kozuleva, M.A., Naidov, I.A., Mubarakshina, M.M. and Ivanov, B.N. (2007) Participation of ferredoxin in oxygen reduction by the photosynthetic electron transport chain. Biophysics 52, 393–397.
[23] Takahashi, M. and Asada, K. (1988) Superoxide production in aprotic interior of chloroplast thylakoids. Arch. Biochem. Biophys. 267, 714–722.
[24] Johnson, T.W., Shen, G., Zybailov, B., Kolling, D., Reategui, R., Beauparlant, S., Vassiliev, I.R., Bryant, D.A., Jones, A.D., Golbeck, J.H. and Chitnis, P.R. (2000) Recruitment of a foreign quinone into the A_1 site of photosystem I. I. Genetic and physiological characterization of phylloquinone biosynthetic pathway mutants in *Synechocystis* sp. PCC 6803. J. Biol. Chem. 275, 8523–8530.
[25] Srinivasan, N. and Golbeck, J.H. (2009) Protein-cofactor interactions in bioenergetic complexes: the role of the A_{1A} and A_{1B} phylloquinones in Photosystem I. Biochim. Biophys. Acta 1787, 1057–1088.
[26] Semenov, A.Y., Vassiliev, I.R., van der Est, A., Mamedov, M.D., Zybailov, B., Shen, G., Stehlik, D., Diner, B.A., Chitnis, P.R. and Golbeck, J.H. (2000) Recruitment of a foreign quinone into the A_1 site of photosystem I. Altered kinetics of electron transfer in phylloquinone biosynthetic pathway mutants studied by time-resolved optical, EPR, and electrometric techniques. J. Biol. Chem. 275, 23429–23438.
[27] Shen, G.Z., Antonkine, M.L., van der Est, A., Vassiliev, I.R., Brettel, K., Bittl, R., Zech, S.G., Zhao, J.D., Stehlik, D., Bryant, D.A. and Golbeck, J.H. (2002) Assembly of photosystem I. II. Rubredoxin is required for the in vivo assembly of FX in *Synechococcus* sp. PCC 7002 as shown by optical and EPR spectroscopy. J. Biol. Chem. 277, 20355–20366.
[28] Farrington, J.A., Ebert, M., Land, E.J. and Fletcher, K. (1973) Bipyridylium quaternary salts and related compounds. V. Pulse radiolysis studies of the reaction of paraquat radical with oxygen. Implications for the mode of action of bipyridyl herbicides. Biochim. Biophys. Acta 314, 372–381.
[29] Allen, J.F. and Hall, D.O. (1974) The relationship of oxygen uptake to electron transport in photosystem I of isolated chloroplasts: the role of superoxide and ascorbate. Biochem. Biophys. Res. Commun. 58, 579–585.
[30] Hiyama, T. and Ke, B. (1971) A new photosynthetic pigment, "P430": its possible role as the primary electron acceptor of photosystem I. Proc. Natl. Acad. Sci. USA 68, 1010–1013.
[31] Khorobrykh, S., Mubarakshina, M. and Ivanov, B. (2004) Photosystem I is not solely responsible for oxygen reduction in isolated thylakoids. Biochim. Biophys. Acta 1657, 164–167.
[32] McConnell, M.D., Cowgill, J.B., Baker, P.L., Rappaport, F. and Redding, K.E. (2011) Double reduction of plastoquinone to plastoquinol in photosystem 1. Biochemistry 50, 11034–11046.
[33] Klughammer, C. and Pace, R.J. (1997) Photoreduction of the secondary Photosystem I electron acceptor vitamin K_1 in intact spinach chloroplasts and cyanobacteria in vivo. Biochim. Biophys. Acta 1318, 133–144.
[34] Guergova-Kuras, M., Boudreaux, B., Joliot, A., Joliot, P. and Redding, K. (2001) Evidence for two active branches for electron transfer in photosystem I. Proc. Natl. Acad. Sci. USA 98, 4437–4442.
[35] Milanovsky, G.E., Ptushenko, V.V., Golbeck, J.H., Semenov, A.Yu. and Cherepanov, D.A. (2014) Molecular dynamics study of the primary charge separation reactions in Photosystem I: effect of the replacement of the axial ligands to the electron acceptor A_0. Biochim. Biophys. Acta 1837, 1472–1483.
[36] Sun, J., Hao, S., Radle, M., Hsu, W., Shelaev, I., Nadtochenko, V., Shuvalov, V., Semenov, A., Gordon, H., van der Est, A. and Golbeck, J. (2014) Evidence that histidine forms a coordination bond to the A_{0A} and A_{0B} chlorophylls and second H-bond to the A_{1A} and A_{1B} phylloquinones in $M688H_{PsaA}$ and $M668H_{PsaB}$ variants of *Synechocystis* sp. PCC 6803. Biochim. Biophys. Acta 1837, 1362–1375.
[37] Redding, K. and van der Est, A. (2006) The Directionality of electron transport in photosystem I in Photosystem I in: Advances in Photosynthesis and Respiration (Golbeck, J.H., Ed.), pp. 413–437, Springer, Netherlands. Vol. 24.
[38] Ptushenko, V.V., Cherepanov, D.A., Krishtalik, L.I. and Semenov, A.Y. (2008) Semi-continuum electrostatic calculations of redox potentials in photosystem I. Photosynth. Res. 97, 55–74.
[39] Shpilyov, A.V., Zinchenko, V.V., Grimm, B. and Lokstein, H. (2013) Chlorophyll a phytylation is required for the stability of photosystems I and II in the cyanobacterium Synechocystis sp. PCC 6803. Plant J. 73, 336–346.
[40] Lefebvre-Legendre, L., Rappaport, F., Finazzi, G., Ceol, M., Grivet, C., Hopfgartner, G. and Rochaix, J.D. (2007) Loss of phylloquinone in *Chlamydomonas* affects plastoquinone pool size and photosystem II synthesis. J. Biol. Chem. 282, 13250–13263.
[41] Lohmann, A., Schöttler, M.A., Bréhélin, C., Kessler, F., Bock, R., Cahoon, E.B. and Dörmann, P. (2006) Deficiency in phylloquinone (vitamin K1) methylation affects prenyl quinone distribution, photosystem I abundance, and anthocyanin accumulation in the Arabidopsis AtmenG mutant. J. Biol. Chem. 281, 40461–40472.
[42] Hosein, B. and Palmer, G. (1983) The kinetics and mechanism of oxidation of reduced spinach ferredoxin by molecular oxygen and its reduced products. Biochim. Biophys. Acta 723, 383–390.
[43] Shinkarev, V.P., Vassiliev, I.R. and Golbeck, J.H. (2000) A kinetic assessment of the sequence of electron transfer from F_X to F_A and further to F_B in photosystem I: the value of the equilibrium constant between F_X and F_A. Biophys. J. 78, 363–372.